

CLAIMS

What is claimed is:

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- 004021-2222222222
- 1 1. A method of identifying an agent that modulates microtubule
2 depolymerization, said method comprising the steps of:
3 (i) contacting a polymerized microtubule with a microtubule severing
4 protein or a microtubule depolymerizing protein in the presence of an ATP or a GTP and said
5 agent; and
6 (ii) detecting the formation of tubulin monomers, dimers or oligomers,
7 wherein the formation of said tubulin monomers, dimers, or oligomers indicates that said
8 agent modulates microtubule depolymerization.
 - 1 2. The method of claim 1, wherein said polymerized microtubule is
2 labeled with DAPI.
 - 1 3. The method of claim 1, wherein said detecting is by fluorescent
2 resonance energy transfer (FRET).
 - 1 4. The method of claim 2, wherein said detecting comprising detecting a
2 change in fluorescence of said labeled microtubule.
 - 1 5. The method of claim 1, wherein said detecting comprises centrifuging
2 said tubulin monomers if present.
 - 1 6. The method of claim 1, wherein said microtubules are stabilized by
2 contact with an agent selected from the group consisting of paclitaxel, a paclitaxel analogue,
3 and a non-hydrolyzable nucleotide GTP analogue.
 - 1 7. The method of claim 1, wherein said microtubule is attached to a solid
2 surface.
 - 1 8. The method of claim 7, wherein said microtubule is attached to said
2 surface by binding with an agent selected from the group consisting of an inactivated
3 microtubule motor protein, an avidin-biotin linkage, an anti-tubulin antibody, a microtubule
4 binding protein (MAP), and a polylysine..

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1 9. The method of claim 1, wherein said a microtubule severing protein or
2 a microtubule depolymerizing protein is selected from the group consisting of a katanin, a
3 p60 subunit of a katanin, an XKCM1, and a OP18 polypeptide.

1 10. The method of claim 9, wherein said microtubule severing protein is a
2 katanin or a p60 subunit of a katanin.

1 11. The method of claim 10, wherein said p60 subunit of a katanin is a
2 polypeptide of claim 26.

1 12. The method of claim 10, wherein said p60 subunit is a polypeptide
2 having the amino acid sequence of SEQ ID NO: 1.

1 13. The method of claim 1, wherein said method is performed in an array
2 where said array comprises a multiplicity of reaction mixtures. each reaction mixture
3 comprising a distinct and distinguishable domain of said array, and wherein said steps are
4 performed in each reaction mixture.

1 14. The method of claim 13, wherein said array comprises a microtitre
2 plate.

1 15. The method of claim 13, wherein said array comprises at least 48 of
2 said reaction mixtures.

1 16. The method of claim 13, wherein said agent is one of a plurality of
2 agents and wherein each reaction mixture comprises one agent of said plurality of agents.

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1 17. A method of identifying a therapeutic lead compound that modulates
2 depolymerization or severing of a microtubule system, said method comprising the steps of:

3 i) providing an assay mixture comprising a katanin p60 subunit and a
4 microtubules;

5 ii) contacting said assay mixture with a test compound to be screened
6 for the ability to inhibit or enhance the microtubule severing or ATPase activity of said p60
7 subunit; and

8 iii) detecting specific binding of said test compound to said p60
9 subunit or a change in the ATPase activity of said p60 subunit.

1 18. The method of claim 17, wherein said detecting comprises detecting
2 ATPase activity utilizes malachite green as a detection reagent.

1 19. The method of claim 17, wherein said p60 subunit is labeled and said
2 test agent is attached to a solid support.

1 20. The method of claim 17, wherein said test agent is labeled and said p60
2 subunit is attached to a solid support.

1 21. The method of claim 17, wherein said microtubules are stabilized by
2 contact with an agent selected from the group consisting of paclitaxel, a paclitaxel analogue,
3 and a non-hydrolyzable nucleotide GTP analogue

1 22. The method of claim 17, wherein said method is performed in an array
2 where said array comprises a multiplicity of reaction mixtures. each reaction mixture
3 comprising a distinct and distinguishable domain of said array, and wherein said steps are
4 performed in each reaction mixture.

1 23. The method of claim 22, wherein said array comprises a microtitre
2 plate.

1 24. The method of claim 22, wherein said array comprises at least 48 of
2 said reaction mixtures.

1 25. The method of claim 22, wherein said agent one of a plurality of agents
2 and wherein each reaction mixture comprises one agent of said plurality of agents

1 26. A polypeptide having microtubule severing activity, said polypeptide
2 comprising an isolated p60 subunit of a katanin, wherein said p60 subunit is encoded by a
3 nucleic acid that hybridizes under stringent conditions with a nucleic acid that encodes the
4 amino acid SEQ ID NO: 1.

1 27. The polypeptide of claim 26. wherein said polypeptide is the
2 polypeptide of SEQ ID NO: 1 or the polypeptide of SEQ ID NO: 1 having conservative
3 substitutions.

- 1 28. The polypeptide of claim 26, wherein said polypeptide comprising at
2 least 8 contiguous amino acids from a polypeptide sequence encoded by a nucleic acid as set
3 forth in SEQ ID NO: 1, wherein:
4 said polypeptide, when presented as an antigen, elicits the production
5 of an antibody that specifically binds to a polypeptide sequence encoded by a nucleic acid as
6 set forth in SEQ ID NO: 1; and
7 said polypeptide does not bind to antisera raised against a polypeptide
8 encoded by a nucleic acid sequence as set forth in SEQ ID NO: 1, that has been fully
9 immunosorbed with a polypeptide encoded by a nucleic acid sequence as set forth in SEQ ID
10 NO: 1.
- 1 29. The polypeptide of claim 26, wherein said polypeptide is the
2 polypeptide of SEQ ID No: 1.
- 1 30. An isolated nucleic acid that encodes a katanin p60 subunit having
2 microtubule severing activity, said nucleic acid comprising a nucleic acid that specifically
3 hybridizes with a nucleic acid that encodes the polypeptide of SEQ ID NO:1 under stringent
4 conditions.
- 1 31. The nucleic acid of claim 30, wherein said nucleic acid encodes a
2 polypeptide of SEQ ID No: 1 or conservative substitutions thereof.
- 1 32. The nucleic acid of claim 30, further comprising a promoter.
- 1 33. The nucleic acid of claim 32, wherein said promoter is a baculovirus
2 promoter.
- 1 34. A kit for screening for agents that modulate microtubule
2 depolymerization, said kit comprising one or more containers containing an isolated
3 microtubule severing protein or a microtubule depolymerizing protein.
- 1 35. The kit of claim 34, further comprising a polymerized microtubule
2 labeled with DAPI.
- 1 36. The kit of claim 34, wherein said microtubule is stabilized by contact
2 with paclitaxel or a paclitaxel derivative.

1 37. The kit of claim 36, wherein said microtubule is attached to a solid
2 surface.

1 38. The kit of claim 37, wherein said microtubule is attached to said
2 surface by binding with a motor protein.

1 39. The kit of claim 34, wherein said microtubule severing protein or
2 microtubule depolymerizing protein is selected from the group consisting of a katanin, a p60
3 subunit of a katanin, an XKCM1, and a OP18 polypeptide.

1 40. The kit of claim 39, wherein said microtubule severing protein is a
2 katanin or a p60 subunit of a katanin.

1 41. The kit of claim 34, wherein said p60 subunit of a katanin is a
2 polypeptide of claim 26.

1 42. The kit of claim 34, wherein said microtubule severing protein or
2 microtubule depolymerizing protein is attached to a solid surface.

1 43. A method of screening for an agent that alters microtubule
2 polymerization or depolymerization or severing, said method comprising:
3 providing labeled tubulin;
4 contacting said labeled tubulin with said agent to produce contacted
5 tubulin;
6 comparing the fluorescence intensity or pattern of said contacted
7 tubulin with the fluorescence intensity or pattern of labeled tubulin that is not contacted with
8 said agent wherein a difference in fluorescence pattern or intensity between the contacted and
9 the not contacted tubulin indicates that said agent alters microtubule polymerization or
10 depolymerization.

1 44. The method of claim 43, wherein said labeled tubulin is in the form of
2 tubulin monomers, tubulin dimers, or tubulin oligomers.

1 45. The method of claim 43, wherein said labeled tubulin is in the form of
2 a microtubule.

1 46. The method of claim 45, wherein said microtubule is attached to a
2 solid surface.

1 47. The method of claim 45, wherein said label is selected from the group
2 consisting of DAPI, ANS, Bis-ANS, ruthenium red, cresol violet, and DCVJ.

1 48. The method of claim 47, wherein said label is DAPI.

1 49. The method of claim 46, wherein said microtubule is attached to said
2 surface by binding with an agent selected from the group consisting of an inactivated
3 microtubule motor protein, an avidin-biotin linkage, an anti-tubulin antibody, a microtubule
4 binding protein (MAP), a polyarginine, a polyhistidine, and a polylysine.

1 50. The method of claim 43 wherein said contacting further comprises
2 contacting said tubulin with a microtubule depolymerizing protein or a microtubule severing
3 protein.

1 51. The method of claim 50, wherein said a microtubule severing protein
2 or a microtubule depolymerizing protein is selected from the group consisting of a katanin, a
3 p60 subunit of a katanin, an XKCM1, and a OP18 polypeptide.

1 52. The method of claim 51, wherein said microtubule severing protein is a
2 katanin or a p60 subunit of a katanin.

1 53. The method of claim 52, wherein said p60 subunit of a katanin is a
2 polypeptide of claim 26.

1 54. The method of claim 52, wherein said p60 subunit is a polypeptide
2 having the amino acid sequence of SEQ ID NO: 1.

1 55. The method of claim 43, wherein said method is performed in an array
2 where said array comprises a multiplicity of reaction mixtures. each reaction mixture
3 comprising a distinct and distinguishable domain of said array, and wherein said steps are
4 performed in each reaction mixture.

1 56. The method of claim 55, wherein said array comprises a microtitre
2 plate.

1 58. The method of claim 55, wherein said agent one of a plurality of agents
2 and wherein each reaction mixture comprises one agent of said plurality of agents.

1 59. The method of claim 43, further comprising listing the agents that
2 alters microtubule polymerization, depolymerization, or severing into a database of
3 therapeutic lead compounds that act on the cytoskeletal system..

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